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ORIGINAL ARTICLE

Reduced parahippocampal and lateral temporal $GABA_A$-[^{11}C]flumazenil binding in major depression: preliminary results

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Abstract
Purpose Major depressive disorder (MDD) has been related to both a dysfunctional γ-amino butyric acid (GABA) system and to hyperactivity of the hypothalamic-pituitary-adrenal axis (HPA). Although GABA has been suggested to inhibit HPA axis activity, their relationship has never been studied at the level of the central $GABA_A$-benzodiazepine receptor in depressed patients or in relation to antidepressant treatment.
Methods Eleven depressed outpatients were compared, before and after treatment with citalopram, with nine age-matched healthy controls. The subjects were scanned using the positron emission tomography (PET) tracer [^{11}C] flumazenil ([^{11}C]FMZ). Parametric voxel-by-voxel Logan plots were compared with methods based on regions of interest (ROI), to provide volume of distribution (V_T) and binding potential (BP_{ND}) values. Plasma GABA levels were determined and a dexamethasone-corticotropin releasing hormone (DEX-CRH) test was performed.
Results In MDD, parametric voxel-by-voxel Logan plots showed bilateral reduced [^{11}C]FMZ binding in the parahippocampal gyrus and right lateral superior temporal gyrus (p uncorrected ≤0.001). In the temporal area, [^{11}C]FMZ binding showed a strong inverse correlation with HPA axis activity. Plasma GABA did not discriminate MDD from controls, but correlated inversely with [^{11}C]FMZ binding in the right insula. Following treatment with citalopram, voxel-based analysis revealed reduced binding in the right lateral temporal gyrus and dorsolateral prefrontal cortex.
Conclusion The bilateral reduction in limbic parahippocampal and right temporal [^{11}C]FMZ binding found in MDD indicates decreased $GABA_A$-benzodiazepine receptor complex affinity and/or number. The inverse relationship between $GABA_A$ binding in the temporal lobe and HPA axis activity, suggests that HPA axis hyperactivity is partly due to reduced GABA-ergic inhibition.

Keywords [^{11}C]Flumazenil · PET · GABA · HPA axis · Depressive disorder

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Introduction

Major depressive disorder (MDD) is a common and disabling disorder. Its final pathophysiological pathway remains unresolved, though increasing evidence points towards a

dysfunctional γ-amino butyric acid (GABA) system [1, 2]. Moreover, in MDD, hyperactivity of the hypothalamic-pituitary-adrenocortical (HPA) axis during an episode is one of the most consistent laboratory findings [3], and has been shown to be related to both its course [4] and treatment outcome [5]. Corticotropin releasing hormone (CRH) neurons in the hypothalamic paraventricular nucleus, the central drive of the HPA axis, receive inhibitory input from GABA-ergic neurons. These neurons may thus provide a structural basis for inhibitory regulation of HPA axis activity [6].

Petty et al. [7] demonstrated that male MDD patients exhibit 10–15% lower total plasma GABA levels than controls. In cerebrospinal fluid, reduced GABA has been found in some studies [8–10], but not in others [11–13]. Using chromatography, Honig et al. [14] found an inverse correlation between depression severity and GABA levels in frontal cortex biopsy tissue of MDD patients resistant to both pharmacotherapy and electroconvulsive therapy (ECT). However, in a follow-up comparison study with controls, Francis et al. [15] found no change in cortical GABA levels.

In post-mortem brain tissue of depressive suicide victims, Korpi et al. [16] found no differences in GABA levels in the frontal cortex, basal ganglia, amygdala and hypothalamus when compared to controls. Early studies, using a [^{3}H]flunitrazepam binding assay, found an increase in the number of $GABA_A$ benzodiazepine binding sites in the frontal cortex of depressive suicide victims, suggesting decreased availability of GABA [17]. No difference in number or affinity of benzodiazepine binding sites has been reported in the amygdala or hippocampus [18].

In vivo, proton magnetic resonance spectroscopy (MRS) techniques have repeatedly shown reduced GABA levels in the dorsomedial and anterolateral prefrontal cortex [1] and in the occipital cortex [19–22] of medication-free unipolar MDD patients. After recovery, prefrontal GABA levels were comparable with those in healthy controls [23], whereas occipital and anterior cingulate cortex GABA levels were still diminished [24, 25]. After 8 weeks of treatment with fluoxetine or citalopram, or a completed course of mood-improving ECT, Sanacora et al. [20, 26] found an increase in occipital GABA levels, but not after 12 weeks of cognitive behavioural therapy, suggesting state- and treatment-type related changes [27].

Using [^{123}I]iomazenil and single photon emission computed tomography (SPECT), Kugaya et al. [22] found no differences in $GABA_A$ benzodiazepine binding between MDD patients and controls. Following ECT, Mervaala et al. [28] found an increase in baseline brain [^{123}I]iomazenil uptake in severe depression, though not in the temporal cortices. Changes in $GABA_A$ binding due to pharmacotherapeutic treatment, other than benzodiazepines, have not been studied.

In summary, data on $GABA_A$ receptor binding in MDD are scarce and conflicting, which may be related to limited resolution and sensitivity of the methods used so far. Therefore, in the present study [^{11}C]flumazenil ([^{11}C]FMZ), a reversible binding central $GABA_A$ benzodiazepine antagonist, was used as a positron emission tomography (PET) tracer for the assessment of $GABA_A$ receptor status. Furthermore, [^{11}C]FMZ binding was studied in relation to GABA levels and HPA axis activity in the peripheral blood of MDD patients before and after treatment with citalopram and of controls, to clarify their mutual relationship. MDD and related HPA axis hyperactivity were hypothesized to be associated with decreased [^{11}C]FMZ $GABA_A$ benzodiazepine binding, which should partly reverse after treatment.

Materials and methods

Subjects

A group of 11 drug-free patients (age 37±11 years, mean ±SD) suffering a current MDD episode were recruited from our outpatient psychiatric clinic. The psychiatric diagnosis was verified using the structured clinical interview for DSM-IV axis I (SCID) [29]. Patients completed the Beck depression inventory (BDI) [30], the Hamilton anxiety rating scale (HAM-A) [31] and the Montgomery Åsberg depression rating scale (MADRS) [32]. A clinical global impression (CGI) [33] scale was completed for each patient by his/her own psychiatrist. Previous psychiatric history included major depressive episode (n=5), dysthymia (n=3), bulimia (n=1) and alcohol dependency (n=2), in nine patients (Table 1). Six patients were completely naive for antidepressants and benzodiazepines. At the time of PET scanning, the patients had to be free of antidepressants for ≥3 months and benzodiazepines for ≥2 weeks.

The patients were age-matched with nine healthy control subjects (age 32±7 years) without current depressive symptoms or a past history of psychiatric illness, as verified by BDI and SCID (Table 1). Exclusion criteria for all subjects included pregnancy, somatic disorders or current use of drugs known to interfere with the GABA-ergic system, including benzodiazepines, psychoactive drugs and alcohol abuse. Written informed consent was obtained from all participants after the procedures had been fully explained. The study protocol was approved by the medical ethics committee of the VU University Medical Center Amsterdam. At baseline, all patients and controls had standard physical and laboratory examinations, including liver and kidney function tests, electrolytes, haematology profile and thyroid function tests. Nine patients completed the treatment phase and were available for posttreatment evaluation.

Table 1 Demographic and clinical characteristics of MDD patients before and after treatment and of healthy controls

Characteristic	MDD patients before treatment (n=11)	MDD patients after treatment (n=9)	p value[a]	Controls (n=9)	p value[b]
Sex (no. F/M)	7/4	6/3		3/6	
Age (years, mean±SD)	37±11	39±11		32±7	
Previous psychiatric history (n)	9			None	
Major depressive episode	5	5			
Dysthymia	3	3			
Bulimia	1				
Alcohol dependency	2	1			
Clinical rating scale scores (mean±SD)					
MADRS	26.9±5.9	10.2±5.9	0.001		
BDI	31.3±7.3	11.7±9.0	<0.001	1.3±1.4	<0.001
CGI	4.4±0.8	2.9±0.8	<0.001		
HAM-A	22.5±5.5	13.7±5.1	0.007		
STAI state	50.2±7.7	38.0±11.6	0.009	30.6±3.2	<0.001

[a] Paired t-test, comparing MDD patients before and after treatment (n=9).

[b] One-way ANOVA, comparing MDD patients before treatment (n=11) and controls (n=9).

Treatment phase

MDD patients were treated with citalopram (dose 33.6± 9.2 mg/day, mean±SD) and supportive counselling (usual treatment) for 8 weeks, starting after the initial dexamethasone suppression-corticotropin releasing hormone stimulation (DEX-CRH) test [34]. Clinical visits took place during weeks 1, 2, 4, 6 and 8. Remission was defined as >50% reduction in the MADRS score and total score of <9.

Scan procedures

The radial artery was catheterized 45 min prior to PET scanning under local anaesthesia (Xylocaine 1%, 1 ml), and contralaterally a venous antecubital catheter was placed in situ. Participants were transferred to the scanner room and studied at rest, in the supine position, with ears unplugged using an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, TN). First, a 10-min 2-D transmission scan was acquired using three rotating ^{68}Ge/^{68}Ga sources, to correct the subsequent emission scan for tissue attenuation. Next, a dynamic 3-D scan (16 frames with progressively increasing frame length) with a total duration of 60 min was acquired, following bolus injection of a mean of 370±45 MBq [^{11}C]FMZ with a specific activity of 62±20 GBq/μmol.

During the scan, arterial whole blood was monitored continuously using an online detection system [35]. Discrete samples were taken at 2.5, 5, 10, 20, 30, 40 and 60 min and these were used for calibrating the (online) blood sampler curve, measuring plasma/whole blood ratios, and determining metabolite fractions, enabling the generation of a metabolite-corrected plasma input curve. Fractional concentrations of hydrophilic metabolites of unchanged (lipophilic) [^{11}C]FMZ were determined by solid-phase extraction of plasma followed by high-performance liquid chromatography (HPLC) [36]. All subjects underwent a T1-weighted structural MRI scan, using a 1.5-T Sonata MR system (Siemens, Erlangen, Germany). All MRI scans were qualitatively normal, as reported by experienced neuroradiologists at the VU University Medical Center. State anxiety scores, as measured by the Spielberger state-trait anxiety inventory (STAI) [37], were obtained before and immediately after the [^{11}C]FMZ scanning session and averaged (Table 1). After 8 weeks of treatment, MDD patients returned for a second [^{11}C]FMZ scan session.

Image processing and analysis

Images were reconstructed using FORE+2D filtered back-projection, applying a Hanning filter with a cut-off at 0.5 of the Nyquist frequency. Images consisted of 63 planes of 256×256 voxels, each 1.2×1.2×2.4 mm, with a reconstructed image resolution of approximately 7 mm full-width at half-maximum (FWHM). The images were analysed with CAPP software provided by the scanner manufacturer (CTI/Siemens, Knoxville, TN) on Sun workstations (Sun Microsystems, Mountain View, CA). MRI scans were coregistered with summed [^{11}C]FMZ images (10–60 min after injection) [38, 39]. Next, regions of interest (ROIs) were manually defined on these coregistered MRI scans. Using the anatomical atlas of Duvernoy et al. [40], ROIs were drawn on consecutive planes in cranial–caudal order, starting with the plane in which the vertical and horizontal diameter of the cerebrum no longer increased and ending with the plane where either the cerebellum or temporal poles were no longer visible. The following structures were selected: anterior, ventrolateral, dorsolateral and orbitomedial prefrontal cortex, anterior and posterior cingulate, medial and lateral temporal lobe, and insular, parietal and occipital areas, cerebellum, hippocampus, putamen, and thalamus. The pons was selected as reference

tissue ROI. ROIs were projected onto the dynamic [^{11}C]FMZ images, generating time–activity curves for each region.

For each study, data were analysed using three different complementary methods, applying both voxel-based and ROI approaches. Parametric volume of distribution (V_T) images were generated using Logan plot analysis with a metabolite-corrected arterial plasma input function [41]; V_T being the ratio of the tracer concentration in tissue to that in plasma at equilibrium. Prior to voxel-by-voxel calculations, reconstructed dynamic [^{11}C]FMZ scans were smoothed using a 10-mm gaussian filter, resulting in an overall image resolution of about 12 mm FWHM. This preliminary smoothing was applied to avoid noise-induced bias of plasma input Logan plot analysis [42]. Consequently, in subsequent SPM analysis, the usual additional smoothing of the parametric data was omitted [43].

For ROI-based methods, which are based on best fits of ROI time–activity curves, V_T, obtained using a single tissue (1T) compartment model with metabolite-corrected plasma input function and an additional parameter for blood volume, was compared with nondisplaceable binding potential (BP_{ND}) values obtained using the simplified reference tissue model (SRTM) [44] with the pons as reference tissue, as validated previously [45]. Here, BP_{ND} is the ratio of receptor density (B_{max}) to radioligand equilibrium dissociation constant (K_D) and is proportional to receptor density *x* affinity [46].

Neuroendocrine assessments

Plasma GABA was measured at baseline screening in both MDD patients and controls. Full blood was centrifuged, plasma collected and frozen at −70°C until the assay of total GABA [47]. DEX-CRH testing was performed at baseline in patients and controls, and repeated in MDD patients after treatment 1 week after the [^{11}C]FMZ scan. Urine (24-h samples) was collected for measurement of free cortisol, with correction for creatinine level. The overnight DEX-CRH test started with the oral intake of 1.5 mg dexamethasone at 2300 hours. The next day at 1400 hours, after a light lunch, 100 µg human CRH (Ferring, Kiel, Germany) was administered intravenously as a bolus via an antecubital catheterized vein. Blood samples for determination of plasma cortisol and adrenocorticotropic hormone (ACTH) were withdrawn 30 min before, and at 0, and 15, 30, 45 and 90 min after injection. HPA axis activity was calculated including cortisol and ACTH time to peak, peak level, area under the time-concentration curve (AUC) and delta value (peak level minus basal level). Intra- and interassay coefficients of variation for urine cortisol were 5% and 9% (RIA, Siemens), for serum cortisol 3% and 6% (Centaur, Siemens), and for ACTH laboratory procedures 3% and 8% (Immulite 2500, Siemens).

Statistical analysis

Demographic, behavioural and endocrinological data were analysed using Statistical Package for the Social Sciences (SPSS) software (version 11.5 for Windows; SPSS, Chicago, IL). One-way analysis of variance (ANOVA) was employed for between-group comparisons of demographic, behavioural, corticosteroid, and molecular binding data. Student's paired *t*-tests were performed for within-group analyses of behavioural and molecular binding data.

Parametric images were analysed using SPM2 (Statistical Parametric Mapping; Wellcome Department of Cognitive Neurology, London, UK). After spatial preprocessing (normalization to anatomical standard space as defined by SPM's Montreal Neurological Institute (MNI) template), images were analysed on a voxel-by-voxel basis with and without proportional scaling. In order to correct for large interindividual global variations, only results for proportional scaling are reported. *P* values are reported at the voxel-level, for *p* uncorrected <0.001, with an extent threshold of ten voxels, unless otherwise specified. Both within- and between-group comparisons were performed in addition to analysis of covariance using clinical rating scale outcomes from MADRS, HAM-A, STAI scores, GABA and corticosteroid data. Additionally, within group correlational Pearson analyses (denoted as *r*) were repeated for these data and ROI V_T or BP_{ND}.

Results

Two pretreatment scans, one posttreatment scan and one control scan could not be included in the final analysis due to scanner breakdown during the session (one), clotting of the arterial line (one) and inconsistencies in the [^{11}C]FMZ metabolite data (two).

Comparison of [^{11}C]FMZ binding in MDD patients and controls

In MDD patients (*n*=9) compared to controls (*n*=8), reduced [^{11}C]FMZ binding was found bilaterally in the parahippocampal temporal gyrus and in the right superior temporal gyrus, using voxel-based SPM analysis (Fig. 1, Table 2). No significant differences in binding were found for any of the ROI-based V_T or BP_{ND} values, nor in relation to clinical parameters.

Comparison of [^{11}C]FMZ binding in MDD patients before and after treatment

Pre- and posttreatment scan pairs from six MDD patients were available for analysis after completion of the 8-week

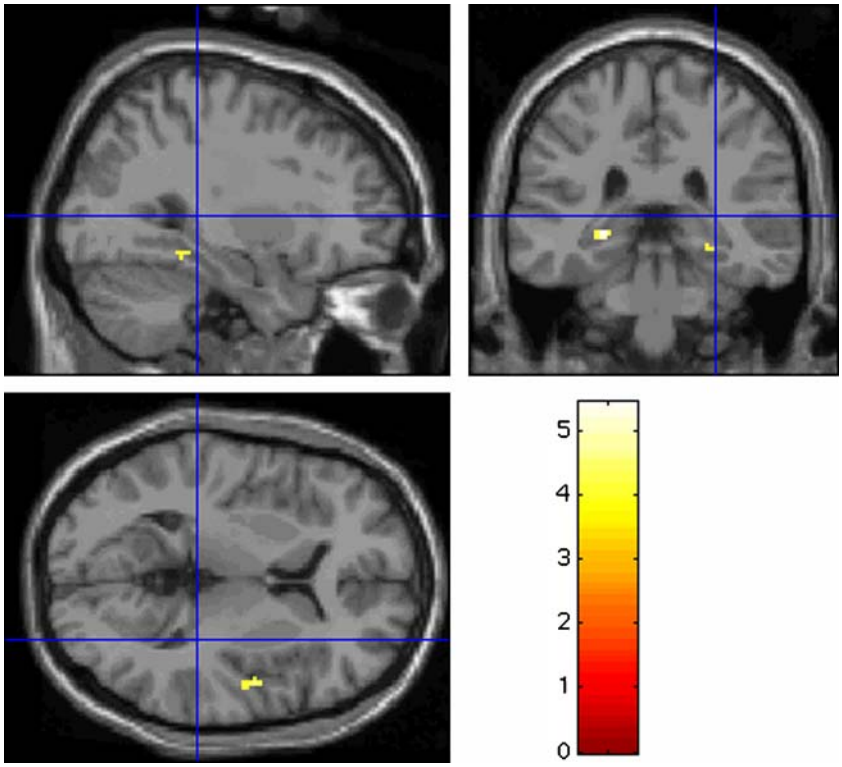

Fig. 1 Statistical parametric map illustrating decreased [^{11}C] FMZ binding in the bilateral parahippocampal gyrus, located at MNI −24, −36, −4 and 30, −38, −12, and the right superior temporal gyrus, at MNI 52, −4, 6, in MDD patients versus controls (p uncorrected ≤0.001, extent ten voxels). *Bottom right* Z-score scale

treatment period. Voxel-based SPM analysis revealed reduced binding after treatment in the right lateral temporal gyrus and dorsolateral prefrontal cortex. There were no significant changes in ROI V_T and BP_{ND} values. V_T pons showed a nonsignificant increase of 6% (V_T 0.90±0.16 to 0.95±0.09, p=0.164).

Clinical rating scales

Although none of the MDD patients was classified with a separate comorbid anxiety disorder, the MDD patients were significantly more anxious than the healthy controls, as measured using the STAI scores, at baseline PET scanning (p<0.001, Table 1). HAM-A scores, including the items depressed mood, tension, fear and somatic anxiety equivalents, decreased significantly (p=0.007), parallel with MADRS and BDI scores (p=0.001 and p<0.001, Table 1). Remission was seen in six out of nine MDD patients.

Neuroendocrine hormones

In MDD patients (n=10), the total GABA level was 1.23±0.14 μmol/l (range 0.971–1.440 μmol/l) compared to 1.00±0.36 μmol/l (range 0.488–1.340 μmol/l) in healthy controls (n=6; p=0.09) and therefore was not discriminative. Plasma GABA could not be recovered in one patient and three healthy controls.

One patient found the DEX-CRH protocol too burdensome and one set of control HPA data could not be acquired for logistic reasons. In the MDD patients (n=10), basal ACTH

Table 2 Brain regions showing reduced parametric [^{11}C] FMZ binding

Condition	K_E[a]	Z-score	L/R	Region	x	y	z
MDD patients < controls	27	3.79	L	Parahippocampal gyrus	−24	−36	−4
	13	3.37	R	Superior temporal gyrus	52	−4	6
	13	3.23	R	Parahippocampal gyrus	30	−38	−12
MDD patients before > after treatment	34	4.63	R	Dorsolateral prefrontal cortex	50	18	8
	11	3.65	R	Lateral temporal gyrus	60	−30	0

p uncorrected <0.001, extent threshold ten voxels.

[a] Number of voxels in cluster.

levels after dexamethasone suppression were significantly increased compared to controls (n=8; p=0.023). The increase in cortisol and ACTH output following additional stimulation with human CRH in MDD patients was not significantly greater than in healthy controls, presumably due to large between-subject variability. Also, before versus after treatment decreases in ACTH and cortisol parameters failed to reach statistical significance (data not shown).

Clinical outcome measures versus SPM [^{11}C]FMZ binding

In the MDD patients, MADRS depression severity scores were inversely correlated with voxel-based [^{11}C]FMZ binding in the right posterior temporal gyrus, bordering the parahippocampal gyrus, and in ventrolateral prefrontal cortex (Table 3). At the group level (MDD patients and controls), but not among the MDD patients, STAI anxiety scores were strongly inversely correlated with [^{11}C]FMZ binding in the left insula and the right temporal gyrus, and bilaterally in the parahippocampal gyrus. HAM-A scores were inversely correlated with [^{11}C]FMZ binding in the right parieto-occipital cortex, adjacent to the parahippocampal gyrus, and in the right dorsolateral prefrontal cortex, adjacent to the superior part of the insula. After treatment, MADRS and STAI scores were no longer significantly correlated with [^{11}C]FMZ binding.

Plasma GABA versus [^{11}C]FMZ binding

In the MDD patients, total GABA was inversely correlated with [^{11}C]FMZ binding in the right insular area proceeding into the temporal lobe, and positively correlated with [^{11}C]FMZ binding in the bilateral anterior cingulate cortex, right posterior cingulate cortex and left temporal gyrus (Table 4).

DEX-CRH outcomes versus [^{11}C]FMZ binding

In the MDD patients, ACTH peak level, delta level, area under the curve and to a lesser extent corresponding cortisol outcomes were inversely related to voxel-based [^{11}C]FMZ binding in the bilateral insular area, extending into the superior temporal lobe (Table 4). A single significant positive correlation was found in the ROI MDD analysis, between V_T right posterior cingulate and peak level cortisol (r=0.814, p=0.049) and cortisol$_{AUC}$ (r=0.860, p=0.028), respectively. A similar trend was seen for V_T left posterior cingulate and cortisol$_{AUC}$ (r=0.791, p=0.061), but not for the identical BP_{ND} ROI. No other ROI was significantly related to DEX-CRH values.

Table 3 MNI coordinates of the inverse interaction between clinical rating scale scores and parametric [^{11}C]FMZ binding

Rating scale	K_E[a]	Z-score	L/R	Region	x	y	z
MADRS[b]	16	4.11	L	Postcentral gyrus	−60	−22	30
	11	3.83	R	Posterior medial temporal/parahippocampal gyrus	38	−36	−18
	14	3.77	L	Precentral gyrus	−48	−22	46
	44	3.67	L	Occipital cortex	−26	−66	−12
	–	3.39	L	Occipital cortex	−20	−74	−8
	17	3.67	L	Parieto-occipital cortex	−14	−66	12
	11	3.52	R	Ventrolateral prefrontal cortex	44	40	−14
HAM-A[b]	44	3.56	R	Dorsolateral prefrontal cortex/superior insula	40	16	12
	95	3.63	R	Parieto-occipital cortex/parahippocampal gyrus	20	−46	4
	10	3.44	L	Parietal cortex	−36	−44	52
STAI[c]	103	3.83	L	Insula	−48	−14	14
	58	3.76	R	Posterior superior temporal gyrus	48	−68	12
	22	3.51	R	Ventrolateral prefrontal cortex	36	22	−16
	13	3.50	L	Dorsolateral prefrontal cortex	−36	46	14
	27	3.39	L	Parahippocampal gyrus	−22	−38	−6
	20	3.36	R	Anterior medial temporal gyrus	52	6	−26
	42	3.27	R	Posterior temporal cortex	28	−50	−12
	–	3.25	R	Parahippocampal gyrus	28	−36	−12

p uncorrected ≤0.001, extent threshold ten voxels.

[a] Number of voxels in the cluster.

[b] In MDD patients.

[c] At the group level; group including all subjects (MDD patients and controls).

Table 4 Interaction between neurohormones and parametric [^{11}C]FMZ binding in MDD patients at baseline

Condition	K_E[a]	Z-score	L/R	Region	x	y	z
Total plasma GABA × [^{11}C]FMZ binding							
Negative correlation	131	4.08	R	Insula	40	-−2	12
Positive correlation	263	4.38	R	Posterior cingulate cortex	12	−44	42
		4.26	R	Posterior cingulate cortex	12	−38	36
	202	4.31	L	Superior/medial temporal gyrus	−46	−24	−10
	98	3.97	L/R	Anterior cingulate cortex	4	40	16
	55	4.39	L/R	Subgenual anterior cingulate cortex	2	52	−14
Output DEX-CRH test × [^{11}C]FMZ binding							
Negative correlation							
ACTH peak level	251	4.43	R	Insula	54	0	−10
	69	4.35	L	Superior temporal gyrus	−42	10	−22
	211	4.23	L	Insula	−54	−8	2
		4.02	L	Insula	−46	−4	4
	60	3.70	R	Medial prefrontal cortex	8	64	10
ACTH delta level[b]	242	4.46	R	Insula	56	2	−8
	194	4.23	L	Insula	−54	−6	0
	52	4.02	L	Superior temporal gyrus	−42	10	−22
	53	3.57	R	Medial prefrontal cortex	8	64	10
ACTH $_{AUC}$	446	4.29	L	Insula	−54	−6	−2
	247	4.03	R	Insula	54	0	−8
	64	3.85	R	Medial prefrontal cortex	8	62	16

p uncorrected <0.001, extent threshold 50 voxels.

[a] Number of voxels in cluster.

[b] Peak level minus basal level.

Discussion

In MDD patients, reduced bilateral [^{11}C]FMZ binding in the limbic parahippocampal temporal gyrus and right lateral superior temporal gyrus was found compared to healthy controls, suggesting decreased $GABA_A$ benzodiazepine receptor affinity and/or numbers. Moreover, in the MDD patients, post hoc voxel-based SPM analysis showed a strong inverse correlation between global [^{11}C]FMZ binding in the bilateral insular–superior temporal area and DEX-CRH-induced release of ACTH and cortisol. This finding supports the hypothesis that a deficient inhibitory GABA-ergic system is related to increased HPA axis activity. Total plasma GABA was not discriminative.

Comparison with previous studies

Our PET findings may be in line with those of a recent study by Aihara et al. [48], showing glucose hypermetabolism in the right parahippocampal gyrus in unmedicated MDD patients. As GABA is a major inhibitory neurotransmitter, decreased binding to the $GABA_A$ benzodiazepine site of the $GABA_A$ receptor may therefore be consistent with loss of inhibition, resulting in increased localized brain activity and metabolic demand. Similarly, Kennedy et al. [49] found diminished glucose uptake in (para)hippocampal regions in depression after successful treatment with paroxetine, suggesting normalization to baseline levels.

Involvement of the insular area in MDD patients, as suggested by the inverse relationship with HPA axis activity found in this study, has been reported in post-stroke depression [50], in panic disorder [51], and more recently by both Cameron et al. [52] and Hasler et al. [53] in panic disorder subjects with comorbid depression. In the MDD patients, total plasma GABA was inversely related to binding in the right insular area, signifying that regionally low $GABA_A$ benzodiazepine binding was related to relatively high available GABA levels.

HPA axis hyperactivity may be due to reduced GABA-ergic tone on the paraventricular nucleus, or alternatively, hyperactivity in the HPA axis may sequentially lead to decreases in $GABA_A$ receptor expression, consistent with animal studies [54, 55]. Interestingly, Merali et al. [56] not only found decreased gene expression for $GABA_A$ receptor subunits in post-mortem brains of suicide victims, but also decreased gene expression for the CRH_1 receptor, suggesting

a downregulation due to increased CRH levels, consistent with increased HPA axis activity in our study.

Psychometrics

MADRS depression severity scores were inversely associated with voxel-based [^{11}C]FMZ binding in areas involved in the pathophysiology of depression and anxiety, that is the right temporal gyrus and the ventrolateral prefrontal cortex, indicating that high depression scores are related to low [^{11}C]FMZ binding [57]. Depression has a close relationship with anxiety, at both the theoretical and clinical level (for review see Kalueff and Nutt [2]). STAI scores significantly differentiated MDD patients from controls. In addition, at the group level, but not in the MDD group, state anxiety was correlated with bilateral decreased parahippocampal [^{11}C]FMZ binding. None of the participants fulfilled the diagnostic criteria for panic disorder or experienced a panic attack in the PET scanner. Most previous studies [51, 52, 58, 59] did not find correlations between anxiety symptom scores and benzodiazepine binding. Only Abadie et al. [60] and Hasler et al. [53] showed correlations in the prefrontal cortex in panic disorder, albeit in the opposite direction. Therefore, the correlation is probably explained by increased anxiousness as part of the depressive syndrome.

After treatment

Following treatment with citalopram, our SPM findings of decreased [^{11}C]FMZ binding in the right dorsolateral prefrontal and temporal cortex may signify either an absolute decrease, or a 'relative' decrease, that is a *lower increase* than in other cortical regions. Increased binding would be in line with findings by Mervaala et al. [28]. Bhagwagar et al. [61] showed that citalopram itself does not exhibit a direct effect upon the $GABA_A$ receptor, though it may indirectly increase the amount of available GABA.

In addition, DEX-CRH-stimulated HPA axis activity partially normalized, although it could not be significantly related to [^{11}C]FMZ binding. The normalization would have been partly mediated by direct effects of citalopram on HPA axis functioning [62].

Methodological limitations

Sample sizes were small, though moderate in the field of PET studies. The study protocol was ambitious, including repeated acquisition of PET data with arterial sampling and neuroendocrine testing, involving a dexamethasone and intravenous CRH challenge. The difficulty in engaging patients was reflected in the time it took to recruit a sufficient number (2 years). Inherent to the diagnosis of depression, the MDD group experienced motivational problems. Groups were matched for age, but not for gender. Previous studies using [^{11}C]FMZ have not revealed gender effects, though this has never been studied in MDD [51–53]. When investigating these modest sample sizes we chose to detect changes with a higher sensitivity, but consequently lower specificity. Given the large number of comparisons, false-positive findings cannot be excluded, and we clearly realize that our results are in need of replication in a larger sample.

Control subjects were tested only once. At retest, depressive patients were less anxious, which may have been due, at least in part, to familiarity with the procedure. This may have influenced PET and HPA axis activity and measurements, although STAI scores at retest were still higher than in healthy controls. Moreover, reduced anxiety in the control group during retest would only have increased the difference with the MDD group.

Voxel-based parametric (SPM) and ROI methods are complementary analytical PET techniques. The main advantage of SPM- over ROI-guided analysis is the fact that all data (voxels) are used. SPM does not require that regions be defined prior to analysis. Therefore, changes that are only present in part of a region, or indeed across regions, can be better identified. However, normalization and smoothing steps reduce its sensitivity. Due to the large number of comparisons made, voxel-based analyses are susceptible to type I errors [63, 64]. ROIs were individually outlined by hand in all subjects and therefore subject to bias. Given that this is a preliminary study, we did not include additional Bonferroni corrections in our ROI analysis to reduce the likelihood of type II error. Although of interest for the suggested relationship between [^{11}C]FMZ $GABA_A$ receptor binding and HPA activity, [^{11}C]FMZ binding in the hypothalamic paraventricular nucleus itself was not quantified, due to its small size and associated partial volume effects.

Conclusion

The main finding of this study is that in MDD patients, FMZ $GABA_A$-benzodiazepine receptor binding is bilaterally decreased and inversely related to HPA axis activity in the limbic temporal areas, suggesting that increased HPA axis activity is partly due to reduced GABA-ergic inhibition.

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